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Faecal microbiology of *Octolasion tyrtaeum*, *Aporrectodea turgida* and *Lumbricus terrestris* and its relation to the carbon budgets of three artificial soils

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With 3 figures

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1. Introduction

Earthworms can significantly alter microbial numbers in soil but reports based on total plate counts of bacteria are conflicting. Some researchers concluded that earthworms increase microbial numbers while others stated the opposite (Day 1950, Parle 1963b, Kozlovskaja 1969, Atlavinytė & Pocienė 1973). Czerwinski *et al.* (1974) suggested that the size of the microbial population prior to invasion of soil by earthworms was a factor in determining the influence of earthworms on microbial numbers. Where microbial populations were high earthworms had little impact on total numbers but where microbial numbers were low earthworms significantly increased them. Additional progress has been made in understanding the relationship between earthworms and microorganisms by examining specific groups of bacteria, rather than total numbers, as well as considering the origin of the sample (faecal pellets vs tunnel linings) (Loquet *et al.* 1977).

Conflicting reports also exist regarding the influence of earthworms on decomposition. It is commonly thought that earthworms accelerate the rate of decomposition. This conclusion is based on observations of reduced surface litter accumulations in the presence of earthworms (Hazelhoff *et al.* 1981, Nielson & Hole 1964, Vimmerstedt & Finney 1973), higher microbial numbers in faeces than associated control soil (Parle 1963b, Day 1950, Kozlovskaja 1969, Ausmus 1977) and lower C: N ratios of faeces than for ingested material (Syers *et al.* 1979; Lunt & Jacobson 1944). Yet contradictory evidence exists in the literature to refute conclusions that earthworms accelerate the rate of decomposition. Atlavinytė (1971) found that *Allolobophora caliginosa* had no effect on the decomposition of straw after 6 months. Stout & Goh (1980) stated surface dwelling species had no impact on reducing accumulation of forest litter. Finally, C: N ratios of earthworm faeces were sometimes higher (Syers *et al.* 1979, Lunt & Jacobson 1944, Watababe 1975) and microbial numbers lower. (Dawson 1947; Nekrasova & Aleksandrova 1976, Atlavinytė & Pocienė 1973) in faeces when compared to control soil.

Obviously a link must exist between the influence of earthworms on microorganisms and decomposition yet few studies have collected both types of data. This study examines the influence of two ecologically distinct earthworm groups on microbial populations and decomposition, as reflected in the carbon and nitrogen dynamics of artificial soils with varying textures.

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2. Materials and methods

Microcosms were created in plexiglass columns using calcareous parent materials from three different textural classes [Sandy Loam (SL); Clay Loam (CL); Silty Clay Loam (SiCL)] and earthworms representing two different ecological groups (SHAW & PAWLUK, 1986). *Lumbricus terrestris* (LINNAEUS) was selected from the anecique group while *Octolasion tyrtaeum* (SAVIGNY) and *Aporrectodea turgida* (EISEN) represented the endogie group (hereafter referred to as geophages). The following combinations were run in triplicate on each soil:

Treatment 1: soil + grass (*Poa pratensis* L./*Festuca rubra* L.)
Treatment 2: soil + grass + *O. tyrtaeum*/*A. turgida*
Treatment 3: soil + grass + *L. terrestris*
Treatment 4: Treatment 2 + *L. terrestris*
Treatment 5: soil alone

The number of earthworm specimens added to each treatment was extrapolated from field population densities reported in the literature. Five specimens with a mean mass of 0.8 g from the endogie group were added to columns in Treatments 2 and 4 and 2 specimens of *L. terrestris* with a mean mass of 2.0 g were added to columns in Treatments 3 and 4. The columns used for Treatments 3 and 4 were taller than those for Treatments 1, 2 and 5 in order to accommodate the vertical tunnelling habit of *L. terrestris*. All columns were maintained under controlled environmental conditions (10 °C/14: 10 day: night cycle) and leached with distilled water on a monthly basis. Soluble organic carbon in the leachates (50 ml) was determined on a Beckman IR Soluble Carbon Analyzer. Additions of 0.5 g (O.D.) of grass were made to Treatments 1—4 on a monthly basis. At the end of one year all columns were stored at temperatures below freezing in order to preserve their chemical and biotical integrity and to provide time for the subsequent dissection of two replicates from each treatment into parts (unaltered soil, tunnel linings, faecal pellets) for chemical and microbiological analyses. Parts were recognized on the basis of optical properties. Faecal material was surficially deposited and/or existed as discrete units in the tunnels. It was characterized by smooth, curved surfaces and a dark brown color due to the presence of humified organic matter. Tunnel linings were dissected out on the basis of location. Their limits were defined by the presence of organic staining. In some cases the tunnel linings were so firmly cemented together they could be easily pulled away from the unaltered soil. The samples were air dried, weighed and ground to pass a 2 mm sieve. Grass remaining on the soil surface was also recovered, dried (60 °C) and weighed.

Microbiological analyses of soil samples from Treatment 1 and the faecal material of *O. tyrtaeum*/*A. turgida* (Treatment 2) and *L. terrestris* (Treatment 3) from all three soil types were conducted using standard serial dilution techniques. Total plate counts of bacteria, actinomycetes and *Cytophaga sp.* were determined on Plate Count Agar (Difco). *Cytophaga* sp. were identified according to the criteria of CHRISTENSEN & COOK (1972). Total plate counts of yeasts and fungi were carried out on Rose Bengal-Streptomycin media (MARTIN 1950). Fungi were differentiated into 3 groups: Non-filamentous, and filamentous with and without dark fruiting bodies. Anaerobes were determined using the Most Probable Numbers (MPN) technique. Dilution-series were incubated in Butlin's broth (BUTLIN *et al.* 1949) with an Fe nail submerged in the broth. Blackening from FeS production was taken as a positive test for the presence of anaerobes.

Enrichments from FVM broth (DOBEREINER *et al.* 1976) were assayed for acetylene reduction to indicate presence of N-fixing bacteria. The enrichments were examined under a phase contrast microscope for identification. Samples from the tunnel linings of *L. terrestris* from all three soil types were also tested for acetylene reduction.

20 isolates of *Cytophaga* sp. from the faeces of *L. terrestris* were assayed for lytic capability on the following media (clearing of the opaque media was taken as a positive test).

Mannan (Difco Yeast Agar)
Chitin a) suspension — commercial chitin pulverized to < 100 mesh and suspended in agar.
b) hydrolyzed chitin (GRAY & BELL, 1962)
c) fungal agar — mushrooms pulverized and suspended in agar

All soil parts dissected from the columns were analyzed for total carbon (Leco Carbon Determinator — Model CR12), carbonate carbon (BUNDY & BREMNER 1972), total nitrogen (determined in a Kjeldahl digest as an ammonia-salicylate complex using a Technicon Autoanalyzer II) and three carbohydrate carbon (neutral sugar) fractions — free, clay-bound, residual (SHAW & PAWLUK 1985). Values for organic carbon were calculated by substaction of carbonate carbon from total carbon.

Two different analysis of variance models were used depending on the type of data under consideration. Whole microcosm data were analyzed as a 3×5 factorial experiment. Data for parts dissected from the columns were analyzed using a split-plot design where the whole plot was a 3^2 factorial with soil type and earthworms at 3 levels and the dissected part was the split-plot factor. Where significant F tests occurred (F-ratio $< 5\%$ probability level) means were compared by the Student-Neuman-Keuls procedure at the 0.05 significance level.

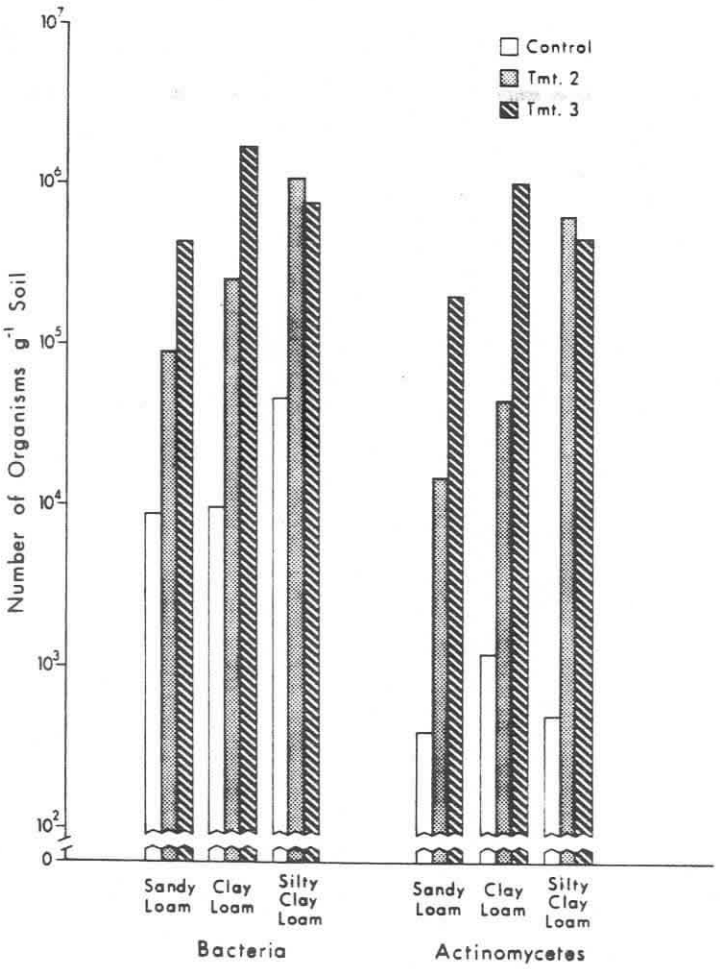

Figure 1. Bacteria and actinomycetes (# g^{-1} soil) in the control (C) and faecal pellets of *O. tyrtaeum/A. turgida* (2) and *L. terrestris* (3).

Calculations for the carbon (C) balances were performed as follows:

1. Carbon Added to Soil From Grass = [Grass added (mg) — Grass recovered after one year (mg)] $\times$ 47/100 (% C of grass).
2. Original Soil Carbon (Non-Extractable — i.e. could not be extracted in neutral sugar analysis) = Soil in Column (mg) $\times$ (% C of soil at beginning of experiment/100).
3. Total Available Carbon = 1 + 2.
4. Final Soil Carbon = [Unaltered Soil (mg) $\times$ % C of unaltered soil/100] + [Faecal Pellets (mg) $\times$ % C of faecal pellets/100) + [Tunnel Linings (mg) $\times$ % C of tunnel linings/100].
5. Net increase (↑) or decrease (↓) in Soil Carbon = 4—2.
6. Carbohydrate Carbon (calculated for each fraction separately — i.e. free, clay-bound, residual) = [unaltered soil (g) $\times$ 40/100 (% C in glucose) $\times$ Neutral Sugar Concentration (NSC) in unaltered soil (mg/g)] + [faecal pellets (g) $\times$ 40/100 $\times$ NSC in faecal pellets (mg/g)] + [tunnel linings (g) $\times$ 40/100 $\times$ NSC in tunnel linings (mg/g)];
   Total Carbohydrate Carbon = Free + Clay-bound + Residual.
7. Non-Extractable Carbon = 4—6 (Total).
8. Leached Carbon = Sum of soluble organic carbon in leachates.
9. Respired Carbon = 3—(4 + 8).
10. Carbon Lost = 8 + 9;

Carbon present in the earthworms biomass was excluded from the carbon balance.

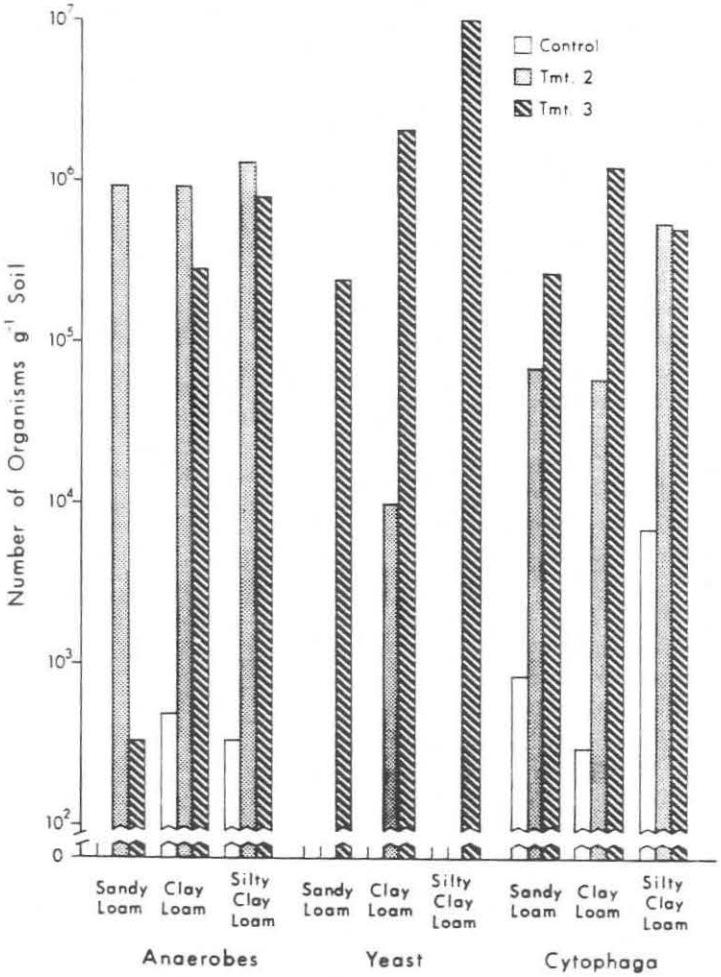

Figure 2. Anaerobes, Yeast and *Cytophaga* sp. (# g^{-1}) in the control (C) and faecal pellets of *O. tyrtaeum/A. turgida* (2) and *L. terrestris* (3).

3. Results

3.1. Microbiological results

Data from the microbiological analyses are presented in Figures 1—3. Total plate counts for bacteria and actinomycetes (Figure 1) are 1—3 orders of magnitude higher in the faecal material than controls for both groups of earthworms and in all 3 soil types. In the SL and CL soils values for *L. terrestris* faecal pellets are generally an order of magnitude higher than for geophages. Data for anaerobes (Figure 2) show that population numbers in the faeces of all earthworms are higher than in the controls. Numbers of anaerobes in the faeces of the geophages always reach a similarly high value (10^6 g^{-1}) regardless of soil type or detection of anaerobes in the controls. Those in faeces of *L. terrestris* reach a value similar to that for the geophage faeces only where anaerobes are also present in controls (CL, SiCL). Yeasts populations are very high (10^5—10^7 g^{-1}) in faeces of *L. terrestris* from all soil types while they are absent in the controls and in geophage faeces from two of the three soils (SL, SiCL) (Figure 2). Numbers of *Cytophaga* sp. are consistently higher in faeces of all earthworms when compared to the controls (Figure 2). Filamentous fungi are higher in number in the faecal material of all earthworms than in controls, but pigmented fruiting bodies are asso-

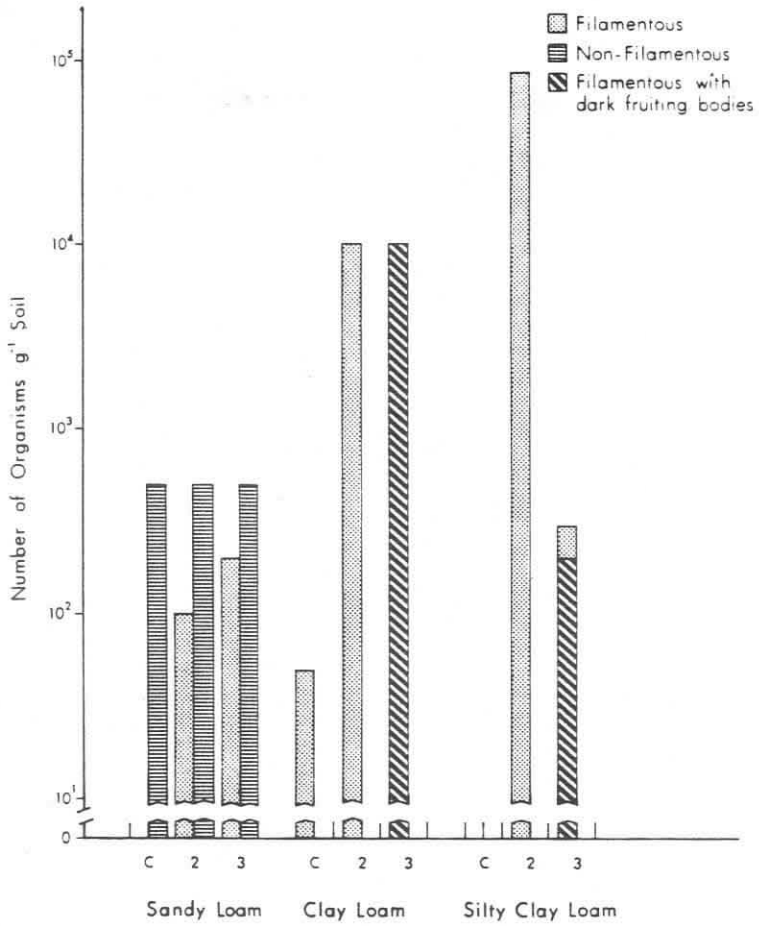

Figure 3. Forms of fungi (# g^{-1} soil) in the control (C) and faecal pellets of *O. tyrtaeum/A. turgida* (2) and *L. terrestris* (3).

ciated only with *L. terrestris* faeces in the CL and SiCL soils (Figure 3). Where non-filamentous fungi are present (SL soil) their numbers are unaffected by earthworms (Figure 3).

A positive test resulted from 35 % of *Cytophaga* sp. isolates assayed against mannan, 55 % assayed on fungal agar and chitin suspension and 40 % assayed on hydrolyzed chitin. Attempts were made to assay enrichments from faecal pellet samples for dinitrogen fixation using an acetylene reduction assay. During the enrichment phase the omnipresence of protozoa in the faecal pellet samples prevented the culture of adequate enrichments for the assay. Subsequently duplicate samples from the tunnel linings of *L. terrestris* were assayed successfully. Ethylene levels of 0.6 and 1.2 μM/24 h were produced from the SL and SiCL soil samples, respectively. The value for the CL soil was particularly high (14.6 μM/24 h). No ethylene was produced by control soil samples. The enrichments assayed were identified as *Spirillum* sp.

3.2. Carbon balances

Only 6 % of the grass added to the columns where *L. terrestris* was present (Treatments 3 and 4) was recovered from the soil surface at the end of a year. Significantly more ($P \leq 0.05$) grass was recovered from columns where the geophages were present (Treatment 2,27 %)

Table 1. Carbon balances — Total Soil Carbon (initial and final values) and Carbon added from grass

Treatment	Soil Texture	Column Number				
		1 Carbon Added to Soil from Grass	2 Original Soil Carbon (Non-Extractable)	3 Total Available * Carbon	4 Final Soil * Carbon	5 Net ↑ or ↓ in Soil Carbon
Control	SL	1,602	1,020	2,622	1,591	↑ 571
Grass Only	CL	1,448	8,590	10,038	8,446	↓ 144
(1)	SiCL	1,214	3,575	4,789	3,903	↑ 328
O. tyrtaeum/	SL	1,970	983	2,953	1,907	↑ 924
A. turgida	CL	1,882	8,366	10,248	7,998	↓ 368
(2)	SiCL	1,726	3,581	5,307	4,240	↑ 659
L. terrestris	SL	2,659	3,717	6,376	6,145	↑ 2,428
(3)	CL	2,182	42,168	44,350	39,329	↓ 2,839
	SiCL	2,197	18,770	20,967	17,732	↓ 1,038
O. tyrtaeum/	SL	2,647	3,678	6,325	5,612	↑ 1,934
A. turgida/	CL	2,264	41,221	43,485	40,919	↓ 302
L. terrestris	SiCL	2,138	18,487	20,625	18,289	↓ 198
(4)						

* see Table 2

Note: Values given in total mg.

Table 2. Carbon balances. Distribution of total available carbon

Treatment	Soil Texture	* Total Available Carbon						
		* Final Soil Carbon						
		Non-Extractable	Carbohydrate Carbon				Carbon Lost	
		Carbon (Net ↑ or ↓)	Total	Free	Clay-bound**	Residual	Leached	Respired**
Control	SL	1,466 (↑ 446)	125	0	0 (0.0)	125	9	1,022 (63.8)
Grass Only	CL	8,141 (↓ 449)	305	0	0 (0.0)	305	8	1,584 (109.4)
(1)	SiCL	3,823 (↑ 248)	80	0	0 (0.0)	80	4	882 (72.6)
O. tyrtaeum/	SL	1,836 (↑ 853)	71	7	28 (1.4)	36	14	1,032 (52.4)
A. turgida	CL	7,602 (↓ 764)	396	32	67 (3.6)	297	7	2,243 (119.2)
(2)	SiCL	4,155 (↑ 574)	85	28	7 (0.1)	50	10	1,057 (61.2)
L. terrestris	SL	5,628 (↑ 1,911)	517	40	456 (17.1)	21	9	222 (8.3)
(3)	CL	37,545 (↓ 4,623)	1,784	433	39 (1.8)	1,312	16	5,005 (229.4)
	SiCL	17,301 (↓ 1,469)	431	254	134 (6.1)	43	9	3,226 (146.8)
O. tyrtaeum/	SL	5,589 (↑ 1,911)	23	9	4 (0.2)	10	8	705 (26.6)
A. turgida/	CL	39,944 (↓ 1,277)	975	394	116 (5.1)	465	15	2,551 (112.7)
L. terrestris	SiCL	17,846 (↓ 641)	443	27	367 (17.1)	49	7	2,329 (108.9)
(4)								

* From Table 1

** value within brackets is carbon for the component expressed as a percentage of carbon added from the grass (Table 1, Column 1)

Note: Values given in total mg.

and from the controls (Treatment 1.43 %). No significant differences in the amount of grass recovered occurred between soil types for the same treatments.

Values calculated for the carbon (C) balances are presented in Tables 1 and 2. Carbon added to the soil from the grass (Table 1) reflects trends for grass recovered. Value for soil carbon and C: N ratios (Tables 3 and 4) are consistently higher for the CL than other soils reflecting the presence of coal flecks in the parent material.

The net increase (↑) or decrease (↓) for soil carbon from initial values to those after the experiment ran one year. are listed in column 5 in Table 1. In the controls a net increase in soil carbon occurred in the SL and SiCL soils while a small net decrease occurred in the CL soil. Where the geophages were active alone (Treatment 2) the trends are similar to those in the controls except the magnitude is greater. Where *L. terrestris* was active alone (Treatment 3) the only net increase in soil carbon occurred in the SL soil, while net decreases occurred in the CL and SiCL soils. The greatest net decrease in soil carbon occurred where *L. terrestris* was active alone in the CL soil. Where both ecological groups of earthworms co-existed (Treatment 4) trends are similar to those where *L. terrestris* was active alone (Treatment 3) except that the net decrease in soil carbon for the SiCL and CL soils is not as great.

Table 3. Comparison of C: N ratios of the unaltered soil from all treatments and soil textures

Soil texture	Treatment Number				
	1	2	3	4	5
SL *(c .18)	de 9.6	de 10.8	de 9.2	de 8.2	e 6.2
CL (a 1.19)	c 17.5	bc 19.6	ab 22.6	a 24.2	a 24.9
SiCL (b .55)	de 10.9	de 10.2	de 11.3	de 9.6	d 12.2

(* value for % O.C. which were significantly different between soil textures but not between treatment)

Note: Values not preceded by the same letters are significantly different $P \leqslant .05$.

Table 4. Comparison of the C: N ratios of the faecal pellets and unaltered soil from Treatments 2—4

Soil Texture	Part	Treatment Number		
		2	3	4
SL	unaltered soil	ab 10.8	a 9.2	a 8.2
	faecal pellets	a 9.1	de 15.8	bcd 13.9
CL	unaltered soil	f 19.6	g 22.6	g 24.2
	faecal pellets	cde 15.0	ef 17.9	cde 15.2
SiCL	unaltered soil	ab 10.2	abc 11.3	ab 9.6
	faecal pellets	ab 10.8	abc 11.3	ab 10.2

Note: Values not preceded by the same letters are significantly different $P \leqslant .05$.

Table 5. Comparison of the % O.C. for the faecal pellets and unaltered soil from Treatments 2—4

Soil Texture	Part	Treatment Number		
		2	3	4
SL	unaltered soil	a .22	a .18	a .16
	faecal pellets	b .84	b 1.28	b .90
CL	unaltered soil	a 1.17	a 1.13	a 1.21
	faecal pellets	b 1.76	b 2.14	b 1.68
SiCL	unaltered soil	a .61	a .50	a .52
	faecal pellets	b 1.51	b 1.78	b 1.98

Note: Values not preceded by the same letters within soil textures are significantly different $P \leqslant .05$.
O.C. ≙ Organic carbon.

Data in Table 2 show the distribution of soil carbon at the end of the experiment (Final Soil Carbon) along with losses of carbon to respiration and leaching. Because carbohydrate carbon is generally a small proportion of total carbon, trends in soil carbon (Table 1) and non-extractable carbon (Table 2) are similar. Exceptions are all treatments for the CL soil and the *L. terrestris*/SiCL combination. In these cases the magnitude of the decrease in non-extractable carbon is always greater than the decrease in soil carbon. No carbohydrate carbon is present in samples from Treatment 5 (soil alone). Where grass was added (Treatment 1) carbohydrate carbon is present only in the residual fraction (Table 2). Where earthworms were present carbohydrate carbon is always present in free and clay-bound fractions in addition to residual fractions (Table 2). Values for total carbohydrate carbon are always highest for the CL soil but the majority of it is in the residual fraction and little is present in the clay-bound fraction. The highest amount and proportion of carbohydrate carbon in the clay-bound fraction occurs where *L. terrestris* was active alone in the SL soil and where both groups co-existed in the SiCL soil. In the SiCL and CL soils loss of carbon to respiration is greater where earthworms were present as compared to the control. The highest losses of carbon to respiration occur in these soils where *L. terrestris* was active. In the SL soil loss of carbon to respiration is the same for the control and where the geophages were active alone, slightly lower than the control where both groups were present and much lower than the control where *L. terrestris* was active alone.

C: N ratios (Table 3) and values for % Organic Carbon (% O.C.) in the unaltered SiCL and SL soils are not significantly different across all treatments. A comparison of data for the unaltered SiCL soil with faecal pellets shows no significant differences between C: N ratios (Table 4) but values for % O.C. (Table 5) are significantly higher for the faecal pellets. A similar comparison for the SL soil shows the C: N ratio (Table 4) of the faecal pellets from Treatment 2 is not significantly different from the unaltered soil although % O.C. is significantly higher (Table 5). For Treatments 3 and 4 in the same soil the C: N ratios and % O.C. are both significantly higher in the faecal pellets than the unaltered soil. C: N ratios of the unaltered CL soil (Table 3) in Treatments 1 and 2 are significantly lower than those for Treatments 3, 4 and 5. In Treatments 2—4 C: N ratios (Table 4) for the faecal pellets are significantly lower, while values for % O.C. (Table 5) are significantly higher, than for the unaltered soil.

4. Discussion

It is often suggested, or implied, in the literature that microbial numbers are high in earthworm faeces due to an increase in availability of organic matter (Bal 1982). Data from this study shows that *L. terrestris* introduced significantly more organic matter into the soil than *O. tyrtaeum*/*A. turgida* but this was not always reflected in higher microbial numbers in their faeces. This is especially true when specific groups of microorganisms are considered (e.g., SL-anaerobes; SiCL-fungi). Frequently microbial numbers in the faeces of the geophages are as high as those for *L. terrestris* even though they introduced less organic matter into the soil.

Possibly the enrichment of microbial populations in earthworm faecal pellets is more closely related to their feeding habits and digestion than their effect on organic matter concentration per se. Many parallels exist between the decomposition of litter digested by ruminants and ruminant-like mammals (Lodha 1974) and earthworms. Physico-chemical characteristics of ruminant faeces which make them a favourable environment for microorganisms (Lodha 1974) also exist in earthworm faeces (Parle 1963b, de Vleeschauwer & Lal 1981, Teotia *et al.* 1950; van Rhee 1969, Bal 1982). Data collected in this study reveal parallels between the faecal microbiology of ruminants described by Lodha (1974) and that of earthworms. These include enrichment of actinomycetes and bacteria, preferential enrichment of *Cytophaga* sp., proliferation of fungi, and enrichment of anaerobic microorganisms, in the faeces of earthworms. More parallels drawn between ruminant and earthworm digestion based on observations in this study can be supported from the literature.

Protozoa, which were omnipresent in the faeces of earthworms in this study, are reported to have a strong association with ruminants (STANIER *et al.* 1970) as well as earthworms (DIXON 1975; PIEARCE & PHILLIPS 1980, MILES 1963). Earthworms ingest one another's faeces and this is accepted as a mode of inoculum transfer amongst ruminants (STANIER *et al.* 1970).

LODHA (1974) described the decomposition of litter digested by ruminant and ruminant-like mammals as a process involving two positive feedback mechanisms. The first takes place in the gut and is dominated by anaerobic decomposition while the second takes place in the faeces and is dominated by fungal activity. Fungi in the faeces produce pigmented spores which disperse readily. Ingestion of these spores can stimulate their germination, thus ensuring fungal proliferation. This mechanism of propagation would be advantagous to fungivorous soil fauna since they feed primarily on vegetative parts (RICHARDS 1974, TWINN 1974). It is proposed that the feeding activities of *L. terrestris* affect fungal dynamics in a similar manner. Fungicide and feeding preference studies (COOK & SWAIT 1975; COOKE & LUXTON 1980; NIKLAS & KENNEL 1981) have suggested the dietary importance of fungi to this animal. Further, HUTCHINSON & KAMEL (1956) demonstrated that fungal spores can remain viable after passage through the gut of *L. terrestris*. In this study enrichment of filamentous forms of fungi with pigmented spores and yeasts occurred in the faeces of *L. terrestris*. Similar enrichments in the faeces of *L. terrestris* were observed by PARLE (1963b). Enrichment of *Cytophaga* sp. in the faeces of *L. terrestris* which exhibited considerable lytic activity against chitin and mannan may assist the earthworm, through a symbiotic relationship, in the digestion of fungi. It has been reported in the literature that in most terrestrial environments fungi constitute the primary decomposers of plant materials (EKLUND & GYLLENBERG, 1974). *L. terrestris* is believed to play an important role in decomposition by fragmenting litter and mixing it into the soil (EDWARDS & LOFTY, 1977). It is proposed that the role of *L. terrestris* in the initial stages of decomposition be extended to include control over fungi.

Just as *L. terrestris* may have more influence than the geophages on fungi, the data indicate that geophages have greater influence on anaerobes. In faeces of *O. tyrtaeum*/*A. turgida* the level of enrichment of anaerobes is the same regardless of soil type or inherent numbers in the controls. *L. terrestris* was only able to enrich anaerobes where they already existed in the control. BAL (1982) stated that geophagous species play an important role in the final stages of decomposition since their activity, dominantly in the mineral soil, leads to homogenization, mineralization and humification of organic matter. Possibly this role is related to their influence on secondary decomposers, and especially anaerobic microorganisms.

Many factors, including the differences between the earthworms' feeding habits and influence on microorganisms, contributed to producing different trends for decomposition and stabilization of organic carbon.

An examination of data for the clay-bound fraction of carbohydrate carbon is important for interpreting the influence of earthworms on the stabilization of organic matter. None of the carbohydrate carbon from any of the controls was in the clay-bound fraction while in all cases where earthworms were present some carbon was extracted in the clay-bound fraction. These data show that earthworms are effective in promoting the stabilization of extractable forms of carbohydrate carbon through binding with clays. The degree to which carbon was stabilized depended on the soil type and species of earthworms involved and how these factors relate to decomposition.

Soil type was a major factor controlling trends for decomposition. In controls where only grass was added (Treatment 1) different trends for the fate of carbon, and therefore decomposition, were established between soil types. Decomposition proceeded most rapidly in the CL soil followed by the SiCL and SL soils. This is supported by the high loss of carbon to respiration and net decline for total soil carbon in the CL soil. In the latter two soils total soil carbon increased and respiration losses were lower. When carbon lost to respiration is calculated as a percentage of carbon additions (grass) the values (Table 2, **) suggest that the potential for decomposition is greater in the SiCL than SL soil. This is supported by data

for the C: N ratios. The differences in trends for decomposition may, in part, be due to the difference between the soils, inherent organic carbon content (Table 3) and therefore their ability to support a viable decomposer community to take advantage of the new source of carbon. However, variations in factors such as moisture retention, aeration, nutrient status and void size and shape are no doubt also important.

The geophages appeared to enhance trends for decomposition already dictated by soil type. This reflects both their feeding habit and influence on microorganisms. The geophages had little contact with the surface litter and were active predominantly in the mineral soil (SHAW & PAWLUK 1986). Decomposition of the surface litter proceeded in a manner similar to the controls since litter was not physically translocated into the mineral soil and hence the geophages did not interfere with the normal activity of fungi which constitute the primary. decomposers. Since the geophages did influence secondary decomposers (e.g. anaerobes, bacteria, *Cytophagas*) the rate of the latter stages of decomposition proceeded more rapidly. In the CL soil the high loss of carbon to respiration relative to additions from the grass, and decline in non-extractable carbon suggests carbon present in the mineral soil prior to the grass additions, was decomposed. This resulted in a net loss of total soil carbon in the CL soil. Decomposition was accelerated by the geophages to a lesser extent in the SiCL than CL soil and remained similar to the control in the SL soil.

L. terrestris played a dominant role in controlling the trends for, as well as the rate of, decomposition. This is attributed to their feeding habit of withdrawing litter from the soil surface and bringing it into intimate contact with the mineral soil and to their influence over fungi (primary decomposers). Despite the addition of more carbon from the grass to the SL soil decomposition was reduced relative to the control (Treatment 1) or where the geophages were active alone (Treatment 2). Evidence for this is the decrease in respiration, greater increase in total soil carbon and higher C: N ratio of ingested material (faeces) where *L. terrestris* was present compared to Treatments 1 and 2. The retarded decomposition in the SL soil is attributed to the inability of *L. terrestris* to propagate the type of fungi which LODHA (1974) described as instrumental to the decomposition process in ruminant faeces. In the same soil *L. terrestris* was also unable to enrich the soil with anaerobes to the level of other soils where earthworms were present and decomposition proceeded more rapidly. The retarded decomposition proved beneficial to the SL soil since more carbon was stabilized in the clay-bound fraction than in any other treatment for this soil. Where *L. terrestris* was active in the CL and SiCL soil decomposition was dramatically accelerated. This enhanced decomposition is attributed to the ability of *L. terrestris* to enrich these soils with anaerobes along with other secondary decomposers, and maintain viable fungal decomposers. Assays for acetylene reduction by *Spirillum* indicate that the potential rate of N-fixation is much greater in the CL than SiCL or SL soils. Possible contributions of soil nitrogen through fixation is important to the greater acceleration of decomposition observed for the CL than SiCL soil in the presence of *L. terrestris*. The higher loss of carbon to respiration in the CL than SiCL soil may also be due to the soils inherent inability to stabilize the more labile (carbohydrate) forms of carbon. The fact more carbohydrate carbon was stabilized in the SiCL soil (31%) than the CL soil (2%) may result from the presence of a higher percentage of smectite in its clay fraction (60%; SANBORN 1981) than the CL soil (33%; ABDER-RUHMAN 1980).

Where both ecological groups co-existed the presence of geophages mollified the dramatic impact of *L. terrestris* on the decomposition process, although the influence of *L. terrestris* still dominated. In the SL soil only a small proportion of the carbohydrate carbon was stabilized and the rate of decomposition was intermediate to those where each group of earthworms was active alone. In the CL soil loss of carbon to respiration is similar to that where the geophages were active alone and much less than where *L. terrestris* was active alone. The concentration of the activity of the geophages in the surficially deposited faeces of *L. terrestris* in this treatment (SHAW & PAWLUK 1985) may account for the higher proportion of carbohydrate carbon stabilized. Since the geophages ingest and re-ingest large quantities of mineral soil they would increase the probability of contact between organic and

inorganic components. Although the stabilization of carbon should be considered a substantial benefit to the CL soil this is also the treatment where adverse soil structure developed (Shaw & Pawluk 1986). Thus, the presence of the geophages alone proved most beneficial to the CL soil since carbon was stabilized but not at the expense of excessive losses of carbon to respiration which occurred where *L. terrestris* was active alone, or adverse soil structure which developed where both groups of earthworms were present. Effects of earthworm activity on decomposition in the SiCL soil were similar to those observed for the CL, however, the SiCL soil had an inherent ability to stabilize carbon, possibly due to the presence of a high percentage of smectite in the clays. As a result the absolute amount and proportion, of carbohydrate carbon stabilized is greater when both groups of earthworms were present in the SiCL than the CL soil.

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6. References

Abder-Ruhman, M., 1980. Mineralogy of soils from east-central Alberta. M. Sc. Thesis. Department of Soil Science. University of Alberta. Alberta. Canada.

Atlavinytė, O., 1971. The activity of Lumbricidae, Acarina and Collembola in the straw humification process. Pedobiologia **11**, 104—115.

Atlavinytė, O., & C. Pocienė, 1973. The effect of earthworms and their activity on the amount of algae in the soil. Pedobiologia **13**, 445—455.

Ausmus, B. S., 1977. Regulation of wood decomposition rates by arthropod and annelid populations In: Lohm, U., & T. Persson (eds.), Soil organisms as components of ecosystems. 6th Int. Coll. Soil Zool., Ecol. Bull. (Stockholm) **25**, 180—192.

Bal, L., 1982. Zoological ripening of Soils. Centre for Agricultural Publishing and Documents. Wageningen. 305 pp.

Bundy, L., & J. Bremner, 1972. A simple titrimetric method for determination of inorganic carbon in soils. Soil Sci. Soc. Am. Proc. **36**, 273—275.

Butlin, K. R., M. E. Adams and M. Thomas, 1949. The isolation and cultivation of sulfate-reducing bacteria. J. Gen. Microbiol. **3**, 46.

Christensen, P. J., & F. D. Cook, 1972. The isolation and enumeration of Cytophagas. Can. J. Microbiol. **18**, 1933—1940.

Cook, M., & A. Swait, 1975. Effects of some fungicide treatments on earthworm populations and leaf removal in apple orchards. J. Hort. Sci. **50**, 495—499.

Cooke, A., & M. Luxton, 1980. Effect of microbes on food selection by *L. terrestris*. Rev. Ecol. Biol. Sol. **17** (3), 365—370.

Czerwinski, Z., H. Jakubczyk & E. Nowak, 1974. Analysis of a sheep pasture ecosystem in the Pieniny Mountains (The Carpathians) XII. The effect of earthworm on the pasture soil. Ekol. Pol. **22** (3), 635—650.

Dawson, R. C., 1947. Earthworm microbiology and the formation of water stable soil aggregates. Soil Sci. Soc. Am. Proc. **12**, 512—523.

Day, G. 1950. Influence of earthworms on soil micro-organisms. Soil Sci. **69**, 175—184.

De Vleeschauwer, D., & R. Lal, 1981. Properties of worm casts under secondary tropical regrowth. Soil Sci. **132** (2), 175—181.

Dixon, R. 1975. The astomatous ciliates of British earthworms. J. Biol. Education **9** (1), 29—39.

Dobereiner, J., I. E. Marriel & M. Nery, 1976. Ecological distribution of *Spirillum lipoferum beijerinck*. Can. J. Microbiol. **22**, 1464—1473.

Edwards, C. A., & J. R. Lofty, 1977. Biology of earthworms. 2nd Edition. A Halsted Press Book. John Wiley and Sons. N.Y.

Eklund, E., & H. Gyllenberg, 1974. Bacter a. In: Dickinson, C., & G. J. Pugh (Eds.), Biology of plant litter decomposition. Vol. 1. Academic press, London and New York. 145 pp.

Grayt, & T. Bell, 1962. The decomposition of chitin in acid soils. In: Doeksen, J., & J. van der Drift (Eds.), Soil Organisms, pp. 222—229.

Hazelhoff, L., P. van Hoof, A. Imeson & F. Kwaad, 1981. The exposure of forest soil to erosion by earthworms. Earth Surf. Processes Landf. **6**, 235—250.

Hutchinson, S., & M. Kamel, 1956. The effect of earthworms on the dispersal of soil fungi. J. Soil Sci. **7** (2), 213—218.

Kozlovskaja, L. S., 1969. Der Einfluß der Exkremente von Regenwürmern auf die Aktivierung der mikroobiellen Prozesse in Torfböden. Pedobiologia **2**, 158—164.

Lodha, B., 1974. Decomposition of digested litter. In: Dickinson, C. H., & G. J. Pugh (Eds.), Biology of plant litter decomposition. Vol. II. Academic press, pp. 213—241.
Loquet, M., T. Bhatnagar, M. Bouché & J. Rouelle, 1977. Essai d'estimation d l'influence ecologique des lombriciens sur les microorganismes. Pedobiologia **17**, 400—417.
Lunt, H., & H. Jacobson 1944. The chemical composition of earthworm casts. Soil Sci. **58**, 367 to 375.
Martin, J. P., 1950. The use of acid, rose bengal and Streptomycin in the plate method for estimating soil fungi. Soil Sci. **69**, 215—232.
Miles, H., 1963. Soil protozoa and earthworm nutrition. Soil. Sci. **95**, 407—409.
Nekrasova, K., & I. Aleksandrova, 1982. Participation of collembolas and earthworms in the transformation of algal organic matter. Sov. Soil Sci. **14** (4), 31—39.
Nielson, G., & F. Hole, 1964. Earthworms and the development of coprogeneous A_1 horizons in forest soils of Wisconsin. Soil Sci. Soc. Am. Proc. **28**, 426—430.
Niklas, J., & W. Kennel, 1981. The role of the earthworm *L. terrestris* (L.) in removing sources of phytopathogenic fungi in orchards. Gartenbauwissenschaft **46**, 3, 138—142.
Parle, 1963a. A microbiological study of earthworm casts. J. Gen. Microbiol. **31**, 13—22.
— 1963b. Micro-organisms in the intestines of earthworms. J. Gen. Microbiol. **31**, 1—11.
Piearce, T., & M. Phillips, 1980. The fate of ciliates in the earthworm gut: an in vitro study. Microb. Ecol. **5**, 313—319.
Richards, B., 1974. Introduction to the soil ecosyste .Longman Group Ltd. U.S.A. p. 106.
Sanborn, P., 1981. Dynamics of a Chernozemic so ystem. M. Sc. Thesis. Department of Soil Science. University of Alberty. Alberta. Canada.
Shaw, C., & S. Pawluk, 1986. The development of soil structure by *Octolasion tyrtaeum*, *Aporrectodea turgida* and *Lumbricus terrestris* in parent materials belonging to different textural classes. Pedobiologia **29**, 327—339.
Stanier, R., M. Doudoroff & E. Adelberg, 1970. The microbial world. Prentice-Hall Inc. pp. 733 to 735.
Stout, J., & K. Goh, 1980. The use of radiocarbon to measure the effects of earthworms on soil development. Radiocarbon **22** (3), 892—896.
Syers, J., A. Sharpley & D. Keeney, 1979. Cycling of nitrogen by surface-casting earthworms in a pasture ecosystem. Soil Biol. Biochem. **11**, 181—185.
Teotia, S., F. Duley & T. McCalla, 1950. Effect of stubble mulching on number and activity of earthworms. University of Nebraska College of Agriculture. Agriculture Experimental Station. Nebraska Research Bulletin 165.
Twinn, D., 1974. Nematodes. In: Biology of plant litter decomposition. Dickinson, C., & G. J. Pugh (eds.). Vol. 1. Academic press, London and New York. pp. 421—465.
Van Rhee, J., 1969. Inoculation of earthworms in a newly drained polder. Pedobiologia **9**, 128—132.
Vimmerstedt, J., & J. Finney, 1973. Impact of earthworm introduction on litter burial and nutrient distribution in Ohio strip mine spoil banks. Soil Sci. Soc. Am. Proc. **37**, 388—391.
Watanabe, H., 1975. On the amount of cast production by the Megascolicid earthworm *Pheretima hypeisnis*. Pedobiologia **15**, 20—28.

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Synopsis: *Original scientific paper*

Shaw, C., & S. Pawluk, 1986. Faecal microbiology of *Octolasion tyrtaeum*, *Aporrectodea turgida* and *Lumbricus terrestris* and its relation to the carbon budgets of three artificial soils. Pedobiologia **29**, 377—389.

The influence of *O. tyrtaeum*/*A. turgida* (geophages) and *L. terrestris* on soil microorganisms and its relation to decomposition in soils from three different textural classes was studied. The influence of *L. terrestris* on decomposition and carbon budgets was linked to its feeding habit and control over primary decomposers (fungi) while the influence of the geophages was linked to secondary decomposers. Stabilization of carbon through binding with clays occurred in all treatments where earthworms were present but not where they were absent. In the silty clay loam (SiCL) and clay loam (CL) soils all species of earthworms accelerated decomposition which was linked to their ability to propagate primary and/or secondary decomposers. This was associated with the stabilization of a much higher proportion of carbohydrate carbon in the SiCL than CL soil which was attributed to the high proportion of smectite clay in the SiCL. In the sandy loam (SL) soil decomposition was significantly retarded where *L. terrestris* was present relative to the control or where the geophages were active alone. This was attributed to the inability of *L. terrestris* to propagate primary and secondary decomposers. The retarded decomposition proved beneficial to the SL soil since a high proportion of carbohydrate carbon was stabilized. Where decomposition was accelerated in this soil, carbon was lost to respiration and little was stabilized.

Key words: Earthworms, microbiology, decomposition, soil texture.